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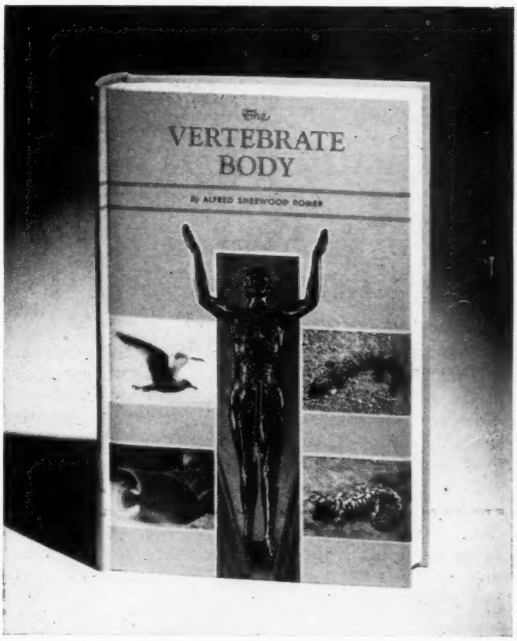

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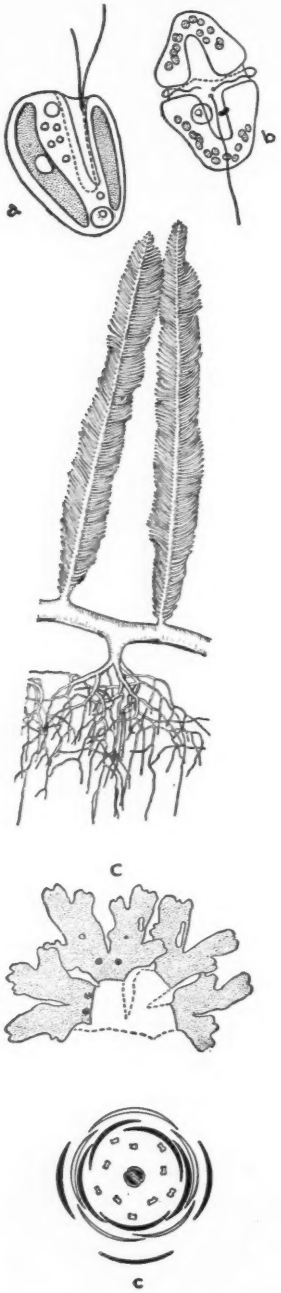

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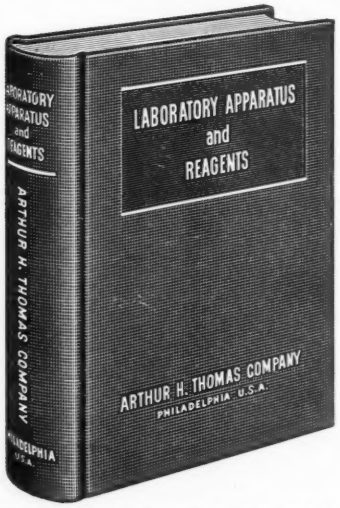

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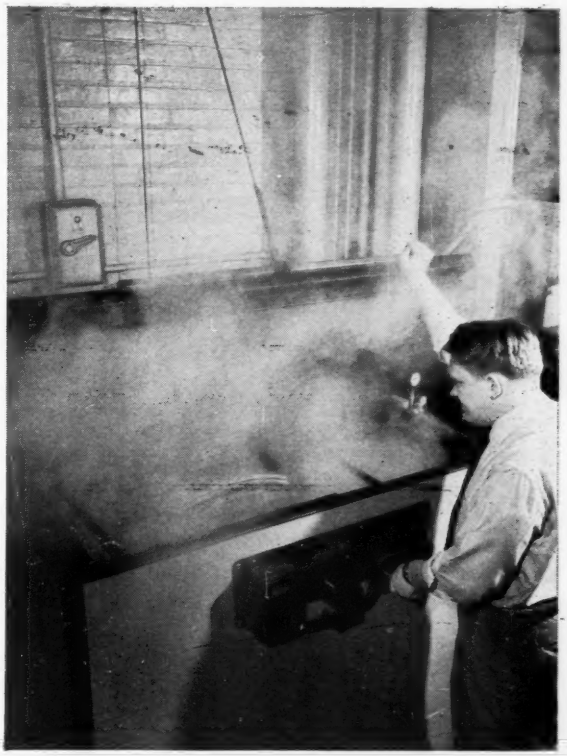

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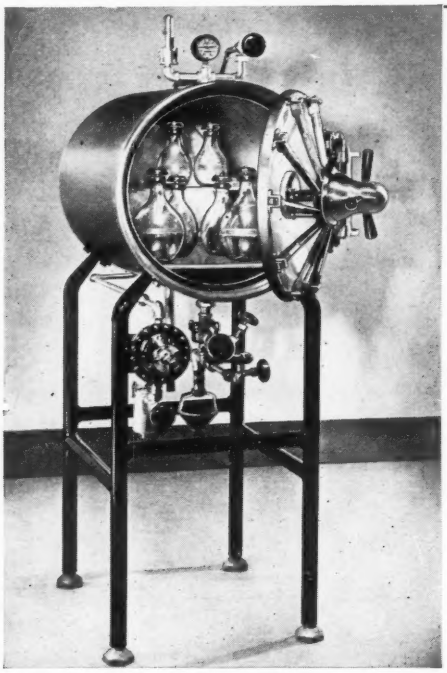

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Bacteriophage: An Essay on Virus Reproduction[1]

S. E. Luria
Indiana University, Bloomington

IN A DISCUSSION of the mechanisms involved in virus reproduction, it is well to start with a critical revision of concepts and definitions, because some of the ideas conceived in other fields have carried into virology implications not justified by the methodology of virus research.

The term *virus* itself can be operationally defined as "an exogenous submicroscopic unit capable of multiplication only inside specific living cells." This definition gives a methodological unity to the field of virology and, by leaving ambiguous two borderline fields—that of obligate parasitic microbes, on the one hand, and that of protoplasmic components transmissible by graft only, on the other hand—suggests some of the possible natural relationships of viruses.

The concept of reproduction requires closer scrutiny. What we observe is the appearance of increased virus activity, associated with an increased number of specific material particles, in a population of virus-infected cells. Virus is produced by the only observable entity, the virus-infected cell, and the mechanism intervening between infection and appearance of the new virus activity cannot be postulated by analogy. In many minds the terms *reproduction* and *self-reproduction* are connected with the idea of increase in size followed by division. Closer scrutiny reveals that increase in size followed by division is bound to be an epiphenomenon of some critical event of reproduction, which must involve point-to-point replication of some elementary structures responsible for the conservation of specificity from generation to generation. Thus, in dealing with cell growth and division we trace the critical event to gene and chromosome duplication. Even a bag of enzymes could grow and multiply only by duplication of discrete enzyme molecules, which can hardly be supposed to grow individually in size and then split. In a repeat, crystal-like structure, such as has been suggested for rod-shaped particles of plant viruses (*2*), the elementary repeated unit must be replicated. In other words, all growth and reproduction should ultimately be traceable to *replication of specific chemical configurations by an essentially discontinuous appearance of discrete replicas.*

One of the first tasks in virus research is to uncover the relation of the virus particle, as we know it in the extracellular state, to what is replicated inside the infected host. Misunderstandings may arise, however, if we fail to distinguish between replication and the more general category of chemical synthesis. There is something peculiar to homologous replication that sets it aside from other types of synthetic reactions. The replication of specific biological units must involve the building of complex specific molecules or molecular aggregates, the only permissible limitation to identity of model and replica being the production of "mutated" structures—the production, that is, of modified elements replicated in the modified form. The fact that the presence of the initial model (gene, virus) is indispensable indicates that this model plays a role in replication; but this role is by no means an obvious one. The model might carry within itself all the enzymes needed for its own synthesis from nonspecific building blocks, or it might act as a directive pattern for synthesis—a pattern in which building blocks are assembled by synthetic enzymes not pertaining to the model itself (this may require a two-dimensional unfolding of the model, to allow point-to-point replication followed by separation of the newly formed unit [*33*])—or it might function as a directive pattern for folding a pluripotential macromolecule into a specific tridimensional replica, possibly with the intervention of a negative template, by analogy with Pauling's theory of antibody formation (*31*).

The study of virus reproduction constitutes one of the best approaches to bridging the gap between growth and replication. I shall deal primarily with the study of bacterial viruses as exemplified by the system of the "T" phages (T1–T7) active on *Escherichia coli* strain B (*5*). Reproduction takes place in a short "latent period" (13 to 45 minutes for different viruses under standard conditions) between the infection of a bacterial cell and its dissolution or lysis, with a rise in phage activity traceable to liberation upon lysis of large numbers of specific phage particles. The number of infected cells, the number of phage particles infecting each cell ("multiplicity of infection"), the time between infection and liberation, and the amount of virus liberated by each cell can be determined accurately (*7*). Moreover, the infecting virus may be "labeled" with easily recognizable properties arising by mutations (*15, 23*). Our

[1] The author's work discussed in this article was supported by grants from the American Cancer Society, recommended by the Committee on Growth.

problem is: How are the newly produced phage particles related to the infecting particles?

On the one hand, the continuity between the two is evidenced not only by the general specificity of reproduction, but also by the observation that mixed infection of a bacterial cell with two closely related phages, such as T2 and its mutant T2r, causes a mixed yield of both infecting types in proportions similar to the ones in the infecting mixture (*15*). The continuity of virus material after infection is also proved by irradiation experiments, which show that the radiation sensitivity of phage inside a bacterium remains the same as that of free phage for a few minutes after infection, indicating that the total amount of radiation-sensitive material remains unchanged (*21, 26*).

On the other hand, there is ample evidence of a deep-reaching alteration of the virus after infection. Doermann, breaking open phage-infected bacteria at different times after infection, found that for several minutes no phage activity could be recovered, and only around the middle of the "latent period" did the first active particles make their appearance (*8*). A similar conclusion had been reached in my laboratory from the study of the effect on phage-infected bacteria of the dye proflavine, which apparently stops phage production, but allows bacteria to lyse and liberate whatever particles were already present when the dye was added (*12*). Thus, there is an "eclipse" of recoverable phage activity between the disappearance of the infecting particles and the appearance of new ones. To the relation between the two I shall return later.

Another line of evidence indicating that the final particles are not a direct product of reproduction of the infecting particles *as such* is the occurrence of complex interactions revealed by experiments with mixed infection. Mixed infection of bacteria with pairs of unrelated phages, such as T1 and T2, or T1 and T7, gives "mutual exclusion": only one type of particle is liberated by each bacterium and the infecting particle of the other type is lost, again suggesting an alteration of the infecting particles (*4, 7*). Phages T2, T4, and T6, however, form a related group and mixed infection of a bacterium with two of them gives rise to a mixed yield. If the two infecting types differ in a character whose alternative forms can manifest themselves in both types—for example, T2r$^+$ and T4r—they give progeny containing, besides the parental types, some new types also, which result from a recombination of the alternative characters r and r$^+$ present in the parental types: T2r$^+$, T2r, T4r$^+$, T4r. This fundamental result, obtained by Delbrück and Bailey (*6*), was greatly extended by Hershey and Rotman (*16, 17*) who, studying recombination among different mutants of phage T2 infecting the same host cells, showed that recombinant types occur with definite specific frequencies for different characters, suggesting a localization of the hereditary properties of bacteriophage in discrete material determinants. We can then, at least formally, interpret recombination experiments according to the model of a phage particle composed of a number of discrete recombinable genetic units, whose number is probably quite large, of the order of 100 or more.

Another kind of interaction is "multiplicity reactivation," which consists in the production of active bacteriophage inside bacteria infected with *two or more* particles of some bacteriophages previously exposed to ultraviolet light and "inactivated"—in the sense that infection of a bacterium with *one* such irradiated particle, while killing the bacterium, would not cause any phage production (*24, 25*). For reactivation to take place, the inactive infecting particles must be of the same type or of genetically related types (T2, T4, T6). This can be interpreted on the basis that reactivation is due to replacement of damaged portions or units of the genetic material of one infecting particle by homologous undamaged portions supplied by the other particles, by the same (unknown) mechanism responsible for the genetic recombinations discussed above. This interpretation of multiplicity reactivation leads to specific expectations concerning the frequency with which bacteria infected with inactive phage particles should produce active phage. On the one hand, the greater the dose of radiation, the smaller should be the frequency of the bacteria that receive two or more particles which can successfully supplement one another, because of more frequent damage in homologous genetic units. On the other hand, for a given dose of radiation, the frequency of bacteria producing active phage should increase with increasing "multiplicity of infection," since this increases the fraction of bacteria that contain mutually supplementing groups of inactive particles. Both expectations are borne out by experiment, and the results agree reasonably well with quantitative expectations derived from a mathematical rationalization of the genetic hypothesis of reactivation. This hypothesis, however, should be considered simply as a working hypothesis until it is substantiated by independent evidence; for the time being it rests mainly on analogy and on a mathematical analysis involving several unproved assumptions. Dulbecco (*10, 11*) has recently discovered in my laboratory that ultraviolet-inactivated phage attached to its host bacterium can be reactivated by exposure to visible light ("photoreactivation," [*20*]). The results of this work may affect, in a way that

is not yet clear, the interpretation of multiplicity reactivation as well.

Be this as it may, the interactions among phage types in mixed infection indicate that in phage reproduction specificity is perpetuated not as a whole, but subdivided into discrete units; we must then look upon these units as the elements whose specific structure is replicated. This does not mean, however, that the units are replicated *separately*: one could imagine that, after the initial eclipse, each new phage particle is produced as a whole and that all recombinations result from late interactions among the newly formed particles. To gain information on this point let us return to the experiments on the kinetics of intracellular phage production.

We have seen that active phage particles appear only around the middle of the latent period; afterwards, their number increases at an approximately linear rate, as shown by Doermann's breakage experiments (*8*). Using mixed infection with different mutants, as in Hershey and Rotman's experiments (*17*), Doermann has recently proved that the very first crop of active particles to appear inside infected bacteria already comprehends the same variety of parental and recombinant types, and in the same proportions (*9*). This indicates that the interactions leading to recombination must take place before or concurrently with the formation of active particles. It seems indeed a reasonable working hypothesis to assume that the active phage particles, which appear at a linear rate in the late phases of intracellular growth, are the end products of reproduction and play no role in further phage production.

This point of view is supported by the following line of evidence. The writer has recently analyzed the production of spontaneous phage mutations during reproduction, and discovered that the new mutants are present in individual bacteria in clones, each clone containing all mutant particles derived from one mutation (*24a*). The distribution of the number of mutants per clone is similar to the one expected from the assumption that the genetic determinants of phage reproduce at a logarithmic rate by successive reduplications, with a constant probability of mutations per reduplication. This suggests the existence of a phase of reproduction of phage in which each new element acts in turn as a source for new replicas.

Does genetic recombination occur either before or during this reproductive phase? Apparently not. In mixed infection experiments (*9, 17*) the particles of any one recombinant type are *not* produced in clones, but are found distributed very nearly at random in individual bacteria. This shows that recombination must follow the logarithmic phase of reproduction, since if recombination occurred earlier each recombinant would give rise to a clone, just as a mutant does. The same experiments also give evidence against recombination's taking place after the formation of the active particles, since it could then occur only by exchange of genetic materials between particles, and the recombinants of reciprocal types should be in equal numbers. Instead, there is no correlation between the numbers of reciprocal recombinants in individual bacteria (*9, 17*).

We conclude, then, that genetic recombination follows the reproduction of the genetic material and does not occur later than the formation of the active particles. It seems probable that recombination and active particle formation occur together, as though the same event that created an active particle also settled its genetic constitution. A hypothesis that fulfills these requirements and that was proposed earlier in a different connection (*21*) is independent replication—and, we should now say, logarithmic replication (*24a*)—of the genetic units composing the phage, followed by their final reorganization into complete, mature phage particles. It should be clearly remembered that no direct evidence for this mechanism of independent reproduction of genetic units has as yet been obtained.

This seems to be as far as we can go at present in analyzing phage production from evidence supplied by the end products. The biochemist has recently thrown some interesting light on phage reproduction, approaching it from the direction of the nonspecific building blocks. The main results (see reference *3*), obtained by determination of total protein and nucleic acids in infected bacteria and by isotope techniques, indicate that the material of the phage particles—which consist entirely or almost entirely of protein and desoxyribosenucleic acid (DNA)—derives in the greatest part from compounds assimilated from the medium after infection. The rate of assimilation of these new materials is similar to the rate of synthesis of bacterial protoplasm in noninfected cells immediately before infection. This suggests that the preexisting synthetic enzymes of the bacterium are responsible and rate-limiting for the formation of the building blocks for phage synthesis. DNA synthesis immediately precedes and parallels the appearance of active phage particles and fails to take place in bacteria infected with inactive, nonreactivated phage T2, which suggests that DNA may be involved mainly in the final steps of the "baking" of active particles.

Failure of phage-infected bacteria to produce specific bacterial components, as distinct from phage substance, is shown by the elegant experiment of Monod and Wollman (*30*) on the absence of adaptive enzyme formation in phage-infected bacteria. A similar failure of enzymatic adaptation has been observed in bacteria infected by ultraviolet-inactivated phage under conditions in which no reactivation occurs (*27*).

A rationale for the suppression by phage infection of specific bacterial syntheses is suggested, in the light of current theories of gene action, by cytological observations (*28*). The first result of infection of a bacterium, with either active or irradiated phage T2, is a rapid disruption of its nuclear apparatus represented by the Feulgen-positive "chromatin" bodies. In the case of infection with active phage, nuclear disruption is followed by the appearance of a granular type of chromatin, which probably represents the new phage itself, as indicated by the failure of this new chromatin to accumulate either after infection with inactive, nonreproducing phage, or after infection with active phage in certain abnormal bacterial strains which upon lysis fail to produce any active phage. These observations suggest that the suppression of synthesis of specific bacterial components in a phage-infected bacterium results from a disruption of the genetic apparatus of the bacterium and its replacement with the genetic apparatus of the virus, resulting in viral rather than bacterial specificity of the protoplasm newly synthesized by the available bacterial enzymatic machinery. The disruption of the genetic apparatus of bacteria infected with inactive phage explains the failure of these bacteria to undergo any further multiplication.

According to this hypothesis, the virus introduces into the host bacterium not only an *additional* organizer of specificity, but a completely *predominant* one, in what could be called parasitism at the genetic level. In the so-called "lysogenic" strains of bacteria, which carry and occasionally liberate phage (*29*), the genetic patterns of host and virus may coexist and function side by side in genetic symbiosis. The difference between phage infection followed by death of the host and phage infection followed by lysogenicity may thus be interpreted as a difference in compatibility relations between the genetic materials of virus and host. The compatibility in lysogenic systems may be more or less stable and its changes may be connected with the sporadic character of phage liberation by lysogenic bacteria (*29*).

Stretching the available evidence, one may construct the following picture of the reproduction of a bacteriophage such as T2: Infection produces a disruption of the genetic organization of the host and a change in the organization of the infecting virus, leading to the formation of a new unit system, the *virus-infected cell,* containing the existing enzymatic machinery of the host and, superimposed upon it, a genetic pattern derived from the virus and directing the synthesis of virus material from nonspecific building blocks. This genetic pattern is resolved into a number of discrete, more or less independent units, the genetic determinants of the virus. The process of formation of the new virus is such that it allows for complex reorganizations to take place and results in the appearance of a population of virus particles that represent the end products of the process as a whole.

This picture, which admittedly has a heuristic rather than descriptive function, presents several major gaps. First, there is a time gap between the disappearance of the initial virus and the appearance of the mature virus. Second, there is a chemical gap between the nonspecific building blocks and the final specific nucleoprotein particles. Third, we have a genetic gap between the genetic determinants of the phage and the phage particle itself, the former being responsible for the inherited specificities, the latter being the carrier of infectivity and, therefore, the only operationally definable unit in the extracellular state. Finally we have a technological gap, in our ignorance of the enzymatic machinery involved in the synthesis of phage from the newly assimilated building blocks. I do not emphasize these gaps in a spirit of pessimism, since it is clear that they involve phases of biological replication about which no biologist possesses any information. The very fact that these gaps can be clearly visualized and delimited in phage analysis suggests that they may be filled more easily by work on bacteriophage than by work on other biological systems.

How far results of phage research can throw light specifically on the events of other virus infections, we do not know. Virus-host relationship may include systems so different that the only similarities to be postulated a priori are those implied in our definition of *virus*. Nevertheless, the picture of reproduction emerging from phage research is likely to bear instructive similarities to other virus infections. Disappearance of recoverable virus activity following infection of a host cell is of general occurrence. Disruption of the genetic apparatus of the host is certainly not general, since cells infected by any one of several plant or animal viruses can still grow and divide. Changes in the synthetic pattern of virus-infected animal cells similar to those of phage-infected bacteria, however, have been recognized (*19*).

Influenza virus in the allantoic membrane of the chick embryo behaves very much like bacteriophage in a culture of a susceptible bacterium, with discrete cycles of intracellular growth and liberation, mutual exclusion in cells infected by two virus strains, and other similarities (*13*); a genetic analysis of this situation would be very desirable. As virus reproduction is apparently more on a level with the reproduction of the genetic material of other cells than with the reproduction of the whole cell itself, it does not seem rash to assume that in all virus infections the material carrying virus activity will be found to be differently organized in its intracellular, replicating, "dynamic" state than in the extracellular, "static" condition. This makes it unlikely that even the most careful and painstaking work on the physical properties of extracellular virus particles (*22, 32*), although very interesting from other points of view, can throw much light on the fundamental problem of virology—virus reproduction. The limitation appears to be an operational one—the alteration, upon infection, of the very properties that the physicochemist analyzes.

In contrast, the limitation of chemical studies on virus-infected cells is merely a technological one—the inadequacy of present-day organic chemistry to deal with the level of organization at which biological specificities are encountered. A sharp refinement of the chemical tool is available, however—immunochemistry. Viruses are good antigens and are in general completely distinct serologically from the uninfected host cells. Can virus specificity be traced serologically during virus reproduction, even in the absence of demonstrable virus activity, to reveal to us the "intermediates" of virus synthesis? The beautiful work of Hoyle (*18*) and of Henle (*14*) on the complement-fixing antigens in the various phases of influenza virus infection shows that these antigens, which carry virus specificity without virus activity, increase in amount *before* virus activity appears. Similar methods now being applied to the study of the early phases of phage production should yield very valuable information.

What will the "intermediates" of phage reproduction, if any, be like? Will they disclose the structure of the hereditary material represented by the postulated genetic units of replication? The recent discovery of an osmotic membrane around the phage particle (*1*) suggests that the latter may consist of both genetic and nongenetic specific materials; caution will be necessary in distinguishing between the two. Nevertheless, it is not unreasonable to hope that this line of work will bring us one step closer to our ultimate goal, the identification of the elementary "replicating units" of biological material and the clarification of their mode of reproduction.

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Technical Papers

Tubular Crystals of Chrysotile Asbestos

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Electron micrographs taken at The Pennsylvania State College and at the National Bureau of Standards show features which indicate that both natural and synthetic chrysotile crystallizes in the form of hollow cylindrical tubes. These bear a striking resemblance to similar crystals of the clay mineral endellite described in 1949 (*2*) by the senior author together with F. A. Hildebrand and Ada Swineford. Fig. 1 is an electron micrograph of halloysite (dehydrated endellite) from Leaky, Real County, Texas. Figs. 2, 3, and 4 are micrographs of

FIG. 1.

chrysotile synthesized by Bowen and Tuttle (*3*) and first studied at high magnification by H. F. McMurdie and M. Swerdlow at the National Bureau of Standards for the Geophysical Laboratory.

Fig. 1 shows (at A) an oblique view of an end section of a tube, (at B) a tube which upon dehydration has split longitudinally and partly unrolled at one end, and (at C) so-called multiple tubes where one cylinder lies within and may project from one or both ends of a larger concentric cylinder.

Fig. 2 shows multiple tubes found in the synthetic chrysotile. The well-defined discontinuities in the concentric layers have not been seen in pictures of halloysite and their significance is not yet understood. Fig. 3 shows multiple tubes which consist of a larger number of concentric cylinders of increasing diameter. Structural control is evidenced by the uniform diameter of a given cylinder and the similar increase in diameter from one cylinder to the next larger one. Fig. 4 shows an interesting conical development of the tubes in addition to longitudinal splitting (A) similar to that seen in Fig. 1 (at B).

FIG. 2.

FIG. 3.

The theory proposed in November 1948 to explain this unusual type of crystal growth in endellite takes into

account the difference in the a_0 and b_0 unit cell dimensions which exists between the silicon-oxygen sheet and the slightly smaller aluminum-hydroxyl (gibbsite) sheet. These sheets fit together to make up the so-called 1:1 structural unit of the clay minerals in the kaolin group. As Pauling (*5*) first pointed out, the slight misfit of the sheets should tend to produce a curvature of the unit in the (001) plane. However, in the mineral kaolinite, $(OH)_8Al_4Si_4O_{10}$, the distance between adjacent 1:1 units is small and the tendency of the sheets to curve is inhibited by the "stretching" effect that the oxygen ions in the silicon-oxygen sheet of one unit have upon the opposing hydroxyl ions in the gibbsite sheet of the adjoining unit. As a result, the typical hexagonal-shaped crystals of this mineral are planar. In endellite, $(OH)_8Al_4Si_4O_{10} \cdot 4H_2O$, on the other hand, because of the presence of interlayer water, the distance between opposing oxygen and hydroxyl ions of adjacent 1:1 units is appreciably greater than in kaolinite. Since the stretching effect is small, the difference in lattice dimensions of silicon-oxygen versus gibbsite sheets exists

FIG. 4.

and curvature results, giving tubular crystals. Measurements of the inner diameters of the tubes pictured in the electron micrographs yield values which are of the same order of magnitude as those obtained by calculations based on the structural considerations. An article describing this research on the kaolin minerals and the resulting structural implications will appear shortly in the *American Mineralogist*.

This structural explanation of the tubular crystals of endellite led to the conclusion that similar conditions might exist in other minerals. The necessary structural requirements are: 1) a sheet structure of the 1:1 type in which one sheet has slightly different dimensions from the other in the plane of the sheet, and 2) bonds between the 1:1 units which are not sufficiently strong to overcome this discrepancy by stretching the ionic lattice on the bottom of one unit to fit the spacing of the ions on the top of the opposing unit. John W. Gruner suggested to the senior author (*4*) that a study of serpentine with the electron microscope might yield significant results. Warren and Hering (*6*) and Aruja (*1*) have shown that antigorite and chrysotile possess layered structures of the 1:1 type. In addition a discrepancy in lattice dimensions exists between the magnesium-hydroxyl (brucite) sheet and the silicon-oxygen sheet which are joined to form the structural unit in these minerals.

Thus a general structural analogy exists between the serpentines and minerals of the kaolinite group. More specifically, differences in the x-ray patterns and dehydration characteristics of kaolinite and endellite are analogous to differences that exist between antigorite and chrysotile.

Electron microscope pictures such as those reproduced here bring out the striking resemblance between the unusual tubular crystals of endellite and chrysotile. Continued research on this problem is being supported by the Office of Naval Research.

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Eperythrozoon suis n. sp. and *Eperythrozoon parvum* n. sp., Two New Blood Parasites of Swine[1]

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A blood parasite of the genus *Eperythrozoon* recently has been observed in swine by Splitter and Williamson (*3*). The organism was found associated with the disease entity of swine known as "ictero-anemia" or "anaplasmosis-like disease."

Animal inoculation studies have established the organism as a new *Eperythrozoon* species, and the name *Eperythrozoon suis* has been given it. Another species has been identified, but observed only under experimental conditions. This organism has been designated as *Eperythrozoon parvum*. David Weinman of the Yale University School of Medicine, and Rue Jensen, Colorado A. and M. College, have concurred in the identity of these organisms as species of the genus *Eperythrozoon*. The presence of two separate species in swine has been established on the basis of differences in morphology

[1] Contribution No. 112, Department of Veterinary Medicine, Kansas State College.

and pathogenicity of the parasites, and by cross-inoculation studies.

E. suis is a large *Eperythrozoon* containing somewhat more chromatin material than other species of this genus. A ring structure which averages about 0.8 μ in diam is the principal form of this extracellular parasite. At the height of parasitic attacks very large ring and discoid forms are present. These vary in size from 1 μ to 2.5 μ. Many of the large ring forms are of distorted shapes, and exhibit an irregular distribution of the chromatin at various points about the ring. Discoid forms appear as flat, solid chromatin masses. Coccus, rod, and various budding forms are also observed. The organisms are usually found upon the erythrocytes, and may be so numerous as to cover the red blood cells completely. Morphological changes occur within a few minutes in citrated or oxalated blood samples. Coccus and rod forms predominate in samples so treated.

E. parvum is observed primarily as small coccus forms and occasional ring structures. The rings are approximately 0.5 μ in diam, and the coccus forms are somewhat smaller. This parasite has displayed a tendency to accumulate in large numbers upon individual erythrocytes, even when very rare in the blood smear. According to Weinman (*4*) this organism is very similar in appearance to *E. dispar*.

Animals used in inoculation studies were held in fly-proof stalls. At least 30 days were allowed following splenectomy or experimental inoculation to determine the presence or absence of parasitic infection. All animals inoculated, with the exception of mice, were injected intravenously with citrated blood.

Four splenectomized pigs which had relapsed with *E. parvum* only, with no ill effects, were injected with blood from a known carrier of *E. suis*. *E. suis* appeared in all four pigs after an incubation period of from two to five days. Acute eperythrozoonosis was evidenced by an acute, febrile, ictero-anemia which developed in each animal. Two subsequently succumbed to the infection. Conversely, two splenectomized pigs that had recovered from acute, clinical infection with *E. suis* were infected with *E. parvum*. A heavy parasitic infection developed, following incubation periods of seven and ten days, but no symptoms or blood damage ensued. One pig known to be susceptible to both parasites was infected with both upon experimental inoculation. In mixed infections *E. suis* has rapidly displaced *E. parvum*.

The two swine species were differentiated from *E. wenyonii* and *E. ovis* by inoculation of heavily infected blood into a susceptible calf and lamb. Both animals remained negative for 30 days, and were then proved susceptible by infecting each with its own *Eperythrozoon* species. Conversely, a susceptible splenectomized pig remained free of parasites following injection of pooled blood heavily infected with *E. wenyonii* and *E. ovis*. Fifty-eight days later the animal was experimentally infected with both *E. suis* and *E. parvum*, the former organism producing an acute ictero-anemia.

Differentiation of the swine species from *E. coccoides* and *E. varians* was accomplished by the intraperitoneal injection of mice with blood heavily infected with the swine parasites. One Swiss white mouse and two local white-footed deer mice of the species *Peromyscus maniculatus*[2] were used, the rodents having first been proved susceptible following splenectomy. All three remained negative for sixty days. Differentiation from *E. dispar* of the vole has not been undertaken. The failure to infect closely related rodents suggests that the *Eperythrozoon* species of swine are not closely related to *E. dispar*.

Splitter and Williamson (*3*) and Splitter (*1*) have demonstrated that *E. suis* is the causative organism of a sporadic disease of swine occurring in the Midwestern United States, which has been known as "ictero-anemia" or "anaplasmosis-like disease." Specific therapy has been obtained with neoarsphenamine (*2*). *E. suis* and *E. parvum* have been found to be common blood parasites of swine in northeastern Kansas.

[2] Identification by H. T. Gier, Department of Zoology, Kansas State College.

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Procedure in Dose Distribution Measurement of 25-Mev X-Rays[1]

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In the preparation of the 25-Mev betatron in the College of Medicine for human therapy (*1*) and animal experimentation, extensive measurements of depth-dose and isodose distributions have been made under a variety of conditions. Two general techniques have been employed, one based on small ion chamber measurements and the other on x-ray film density response.

The ionization measurements were made in a water-filled phantom shown in Fig. 1. This phantom had a front window of taut Nylon sheet (0.003 in. thick) to facilitate measurements close to the surface. The position of the ion chamber in the phantom was remotely controlled by selsyn motors from the control room of the betatron building. The driving selsyns in the control room also acted as indicators of the precise location of the ion chamber. Size No. 1 selsyns have proved entirely satisfactory in operation and precisely position the ion chamber to within ½ mm of the position indicated in the control room. Cylindrical and flat ion chambers have been employed. The diameter and height dimensions of the cylindrical chamber were 5 mm, with a central collection

[1] This work was supported in part by a grant from the American Cancer Society.

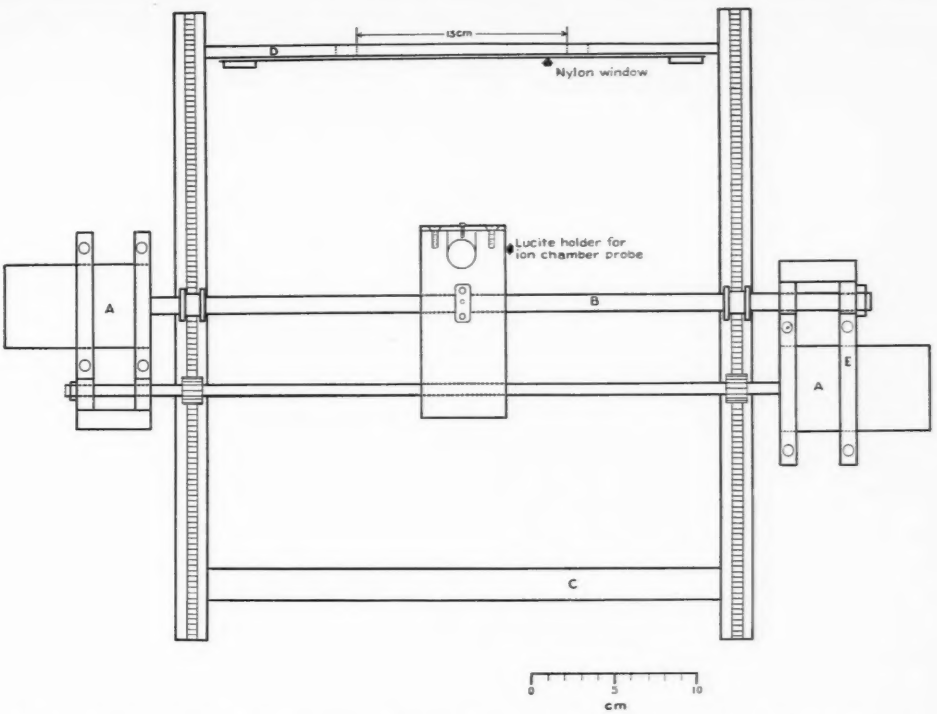

FIG. 1. Remote-controlled water phantom. A denotes selsyn motors; B, square-thread lead screw; C, wooden sides; D, front surface supporting thin Nylon windows; E, aluminum mounting brackets.

electrode of graphite rod. The flat chambers had a thin Nylon front surface (0.003 in.) coated with Aquadag. The collecting electrode consisted of a thin carbon disk 5 mm in diam located 1.5 mm behind the Nylon surface. The volume of these chambers was about 30 mm³. A conventional inverse feedback, direct current amplifier was used, employing the Victoreen VX-41A as a cathode follower in the first stage. The amplifier output was observed directly on a large microammeter, or was further amplified and automatically registered on a graphic recorder. Linearity and reproducibility tests indicated an uncertainty of not greater than 1% in dosage measurement. With this amplifier and remote-controlled arrangement[2] a complete isodose distribution could be obtained in a single uninterrupted operation of the betatron.

A series of measurements of the dose-density relation of industrial x-ray films established a linear relation $r = mD$ for type A and type M films. D refers to the net density measured with an electronic densitometer employing a photocell and amplifier, and r refers to the dose as recorded by a Victoreen thimble chamber. For type A film $m = -0.27t + 8.88$, where t is the developer temperature in the 20° C to 25° C range. For type M film $m =$ 7.09 at 23° C. Kodak rapid developer was used with a development time of 5 min. Great care in the control of temperature, development time, and developer condition was needed to obtain reproducible results.

For relative dose distribution measurements the x-ray films were inserted between 3.2-mm-thick sheets of a Presdwood phantom (density 1.02). The phantom was sealed in a light-tight box with a front surface of paper 0.001 in. thick. All films in a single exposure were developed simultaneously. Fig. 2 displays the experimental depth-dose points obtained in a single film phantom exposure. The x-ray beam was collimated to a circular cross section of 5-cm diam at 80 cm skin target distance. The first 5 cm of the solid curve is based on four sets of data. The vertical dashed lines represent maximum deviations of three depth-dose curves obtained with a flat ion chamber in the water phantom. The skin dose appears to be about 8% of the maximum dose on the basis of film measurements. Ion chamber measurements commence at 0.25-cm depth and average higher than the film data. In the region where the dose gradient is less steep the two methods agree more closely.

In Fig. 3 is shown an isodose distribution obtained in a single continuous operation of the betatron with a flat ion chamber in the water phantom. These data were taken with a beam 10 cm in diam at 80-cm skin target

[2] The remote-controlled water phantom was designed in May, 1948, when the treatment of a patient with the betatron in Champaign (*1*) showed need for such apparatus.

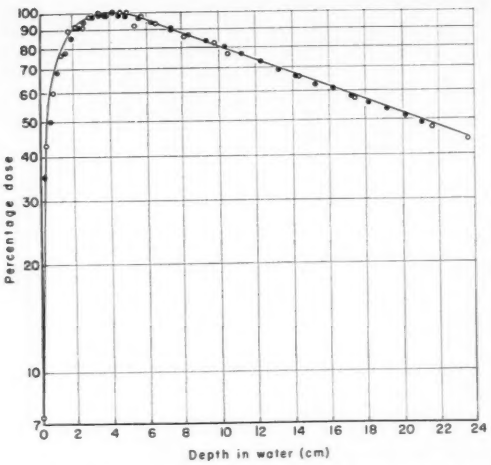

FIG. 2. Depth-dose distribution. Solid points are data obtained with ion chamber in water phantom with 5-cm field at 80-cm skin target distance. Hollow points are data obtained with a film phantom exposed under the same conditions.

distance. The surfaces were shaped by the use of a differential absorber in the x-ray beam consisting of a conical ensemble of fiber disks. No deviation greater than 2½% from perfectly flat isodose surfaces exists. The average of more than one ion chamber or film determination yields even flatter surfaces than those shown. The intensity of radiation 5 cm outside the geometrical edge of the beam is less than 1% of that within the beam.

The depth-dose distribution obtained with a 1-cm-diam beam falls off more rapidly at depths beyond 5 cm than with beams of larger cross-sectional area. The dependence of depth-dose on beam area is much less than the strong dependence which characterizes lower energies (*2*). A detailed analysis of these data is in preparation.

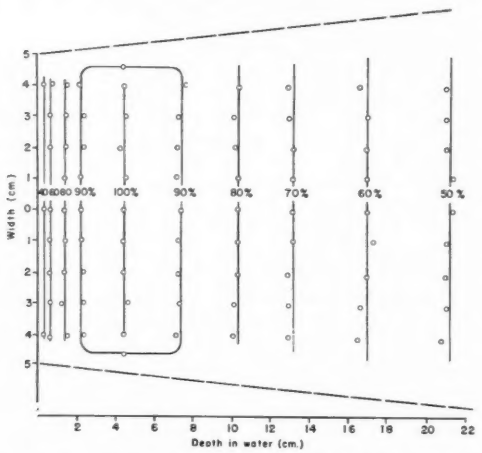

FIG. 3. Isodose distribution obtained in single operation of betatron with ion chamber in remote-controlled water phantom. Beam 10 cm in diam at 80-cm skin target distance filtered with hydrocarbon absorber.

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Antifolliculoid Activity of Vitamin A[1]

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Continued cornification of vaginal epithelium is considered to be a reliable early manifestation of vitamin A deficiency in female rats (*1*). Sherwood *et al.* (*9*, *10*) reported that feeding large amounts of carotene suppressed the estrous vaginal smear picture in the normal rat; however, other workers administered high doses of vitamin A orally (*3*), or by injection (*2*), and found no inhibition of the vaginal response to estrogen. Recent studies on mice (*11*) tend to corroborate the observations of Sherwood *et al.*

Inasmuch as the liver is known to store and inactivate most of the ingested vitamin A (*5*) and probably much of the injected material, we doubted that these methods would result in making available a really high level of vitamin A to any particular target organ. A series of experiments have been conducted involving the intravaginal instillation of various substances by the method employed by Krichesky and Glass (*6*).

Bilaterally ovariectomized rats of an inbred strain were estrogenized by subcutaneous implantation of α-estradiol pellets.[2] After estrous (cornified) smears were obtained from the vagina, sesame oil, cod liver oil, and vitamin A alcohol in sesame oil and in acetone-sesame oil[3] were applied intravaginally in 0.05-ml daily doses, using a blunt 18-gage needle on a tuberculin syringe. Vaginal smears, taken daily during and subsequent to the period of administration of oil solutions, were stained with Wright's stain. The vaginal pictures were classified according to the dominant cell types seen: leukocytes, nucleated epithelium, nucleated vacuolated epithelium, precornified cells (i.e., with nuclear remnants), and cornified cells.

Table 1 demonstrates that topically applied vitamin A results in definite alteration of the keratinized picture produced by estrogen. The appearance of ovoid and round cells with vesicular nuclei, including many with large vacuoles in their cytoplasm, characterizes most smears after two or more days' treatment with vitamin A (Fig. 2). Some cornified and precornified cells were

[1] Aided by research grants from the University of California Board of Research.

[2] Generously supplied by Dr. W. H. Stoner, Schering Corporation, Bloomfield, N. J.

[3] Prepared from crystalline material produced by Distillation Products Industries, generously supplied by Dr. Philip L. Harris and C. J. Staud, Eastman Kodak Co., Rochester, N. Y.

TABLE 1

EFFECT OF INTRAVAGINALLY APPLIED VITAMIN A SOLUTIONS ON THE ESTROGENIZED VAGINAL SMEAR TYPE

Animal group	Treatment	No. of animals	Days of treatment	Total dosage in ml	No. of animals with vaginal smear type :*		
					L, NVE, NE	NE, PC, C	PC, C
Ia	Cod liver oil (780 I.U./ml)	10	2	0.10	0	0	10
	Sesame oil	4	2	0.10	0	0	4
Ib	Cod liver oil	10	16	0.80	0	2	8
	Sesame oil	4	16	0.80	0	0	4
IIa	Vit. A in acetone-sesame oil (25,000 I.U./ml)	17	4	0.20	14	3	0
	Acetone-sesame oil	6	4	0.20	0	1	5
IIb	Vit. A in sesame oil (30,000 I.U./ml)	20	6	0.30	15	5	0
	Sesame oil	10	6	0.30	0	1	9
IIc	Vit. A in sesame oil (50,000 I.U./ml)	10	8	0.40	7	3	0
	Sesame oil	4	8	0.40	0	1	3

* L = leukocytes, NE = nucleated epithelium, NVE = nucleated vacuolated epithelium, PC = precornified cells, C = cornified cells.

also generally present in these smears, but in greatly reduced numbers. The rats treated with sesame oil, on the other hand, showed no appreciable inhibition of the estrogen-induced keratinization (Fig. 1).

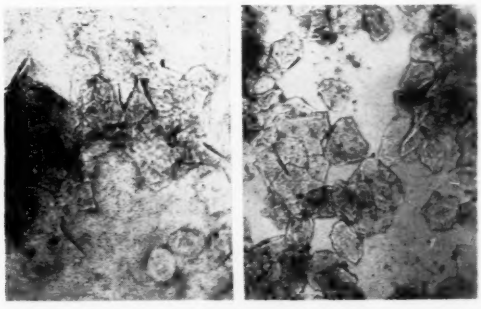

FIG. 1. *Left*, vaginal smear from estrogenized castrated rat (group IIb), before administration of sesame oil (Wright's stain. Magnification 87 ×). *Right*, vaginal smear from same animal, after intravaginal application of 0.05 ml sesame oil daily for 6 days (Wright's stain. Magnification 87 ×).

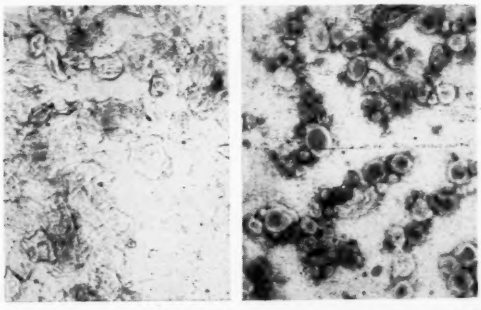

FIG. 2. *Left*, vaginal smear from estrogenized castrated rat (group IIb), before administration of vitamin A (Wright's stain. Magnification 87 ×). *Right*, vaginal smear from same animal, after intravaginal application of 0.05 ml vitamin A alcohol in sesame oil (1500 I.U.) daily for 6 days (Wright's stain. Magnification 87 ×).

The absence of a definite effect from cod liver oil is probably due to the relatively small amounts of vitamin A available in the doses administered, and possibly to the nonspecific keratinization-favoring effect of certain materials, such as cholesterol (*6*), present in cod liver oil.

The data herein presented may support the contentions of some earlier investigators (*4*, *8*) who related all epithelial metaplasia and keratinization, at least in the rat, to the same basic etiologic factor (local vitamin A inadequacy), regardless of the initiating agent. The general metabolic role of vitamin A in favoring the synthesis by epithelial cells of glycoproteins (mucin) over fibrous proteins (keratin), as suggested by Mason and Ellison (*7*), is further indicated here.

A histologic study of the vaginal reaction to various locally applied oil solutions, including vitamin A in oil, along with data supplementary to those summarized herein, will be reported elsewhere.

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Effects of 2,4-Dichloro-5-iodophenoxyacetic Acid and Its Derivatives as Plant Growth Regulators

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Radioactive iodine has been useful in studying the absorption and translocation of growth-regulating substances by plants (*2, 4*). Work in this field has thus far been limited to the use of radioactive 2-iodo^{131}-3-nitrobenzoic acid. A series of growth-regulating substances of the phenoxy type closely related to 2,4-dichlorophenoxyacetic acid (2,4-D)[1] have now been synthesized and some

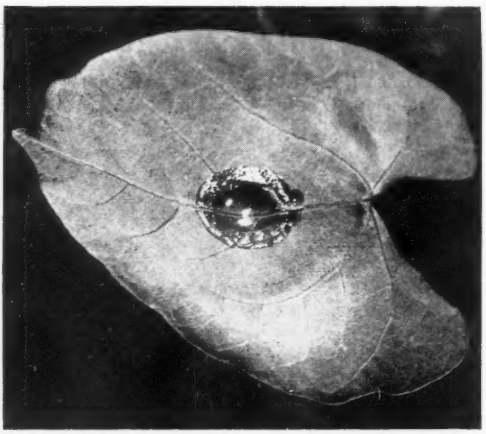

FIG. 1. Method of treating a known area of leaf with a measured amount of liquid containing a plant growth regulator, by enclosing the area with a lanolin ring.

of them tagged[2] with I^{131} (*3*). These new compounds consist of 2,4-dichloro-5-iodophenoxyacetic acid (2,4-D-5-I) and 14 derivatives. They offer promise of extending the usefulness of radioactive isotopes in learning how growth regulators are absorbed and translocated, and in studying the physiological mechanism involved when plants respond to these chemicals.

Plant responses to stable 2,4-dichloro-5-iodophenoxyacetic acid and its derivatives are described here, and their effects are compared with those resulting from the use of an equimolar amount of 2,4-D. The use of 2,4-D-5-I^{131} and its derivatives as radioactive tracers will be described in a later paper.

2,4-Dichloro-5-iodophenoxyacetic acid was applied separately to the leaves, stems, and roots of kidney bean plants. As controls, comparable plants were similarly treated with an equimolar amount of 2,4-D. Both 2,4-D-5-I and 2,4-D brought about stem curvatures when applied to three plant parts. It was evident that 2,4-D-5-I was absorbed at about the same rate as was the 2,4-D, whether applied to the leaves, stems, or roots.

Translocation of 2,4-D from leaves to stems of bean plants is associated, either directly or indirectly, with the translocation of carbohydrates from the leaves. Tests were made to determine whether the translocation of 2,4-D-5-I from leaves was also related to the movement of photosynthate. In this experiment, a ring of lanolin was stamped on the upper surface of the primary leaves of bean seedlings. During this stage of development, carbohydrate reserves in leaves of bean seedlings are relatively low (*1*). The ring was placed along the midrib and approximately 1 cm from the petiole attachment. Approximately 0.5 cm^2 of leaf surface was thus enclosed. One-hundredth of a milliliter of 95% ethyl alcohol containing 2% of Tween 20 as a carrier and 10 μg of 2,4-D-5-I was applied evenly to the enclosed leaf area with the aid of a 0.1-ml pipette (Fig. 1). Other comparable leaves were treated in a similar manner with an alcohol-Tween mixture containing an equimolar amount of 2,4-D. In this way, the growth regulators were confined to a known position on each leaf and covered a known area. Following treatment, the alcohol evaporated rapidly, leaving a thin layer of Tween 20 in which the growth regulator was dissolved. Control leaves treated in a similar manner with the alcohol-Tween mixture alone showed no injurious effects.

TABLE 1

COMPARISON OF THE INHIBITING EFFECT OF 2,4-D-5-I WITH THAT OF 2,4-D WHEN APPLIED TO LEAVES

Treatment	Percent reduction in terminal bud growth
2,4-Dichlorophenoxyacetic acid	
Untreated control	
0.5 μg per 0.01 ml	38
1.0 " " " "	66
2.0 " " " "	80
3.0 " " " "	91
2,4-Dichloro-5-iodophenoxyacetic acid	
2.0 μg per 0.01 ml	11
5.0 " " " "	18
10.0 " " " "	33
20.0 " " " "	57
40.0 " " " "	77

Following treatment, one half of the plants treated with 2,4-D-5-I and half of those treated with 2,4-D were placed in darkness, and the remaining plants of each group were grown under artificial illumination. Neither 2,4-D-5-I nor the 2,4-D was translocated from the leaves of the plants kept in darkness. Both compounds were translocated readily from leaves of illuminated plants as was indicated by the development of marked stem curvature. These results indicate that the translocation of both 2,4-D-5-I and 2,4-D from leaves took place under conditions favorable for the production and translocation of photosynthate.

2,4-D-5-I and 2,4-D were compared for growth-inhibiting effect. Various concentrations of each acid were ap-

[1] The abbreviation 2,4-D is used to denote only the parent acid.

[2] Radioactive 2,4-D-5-I^{131} was originally synthesized in the Bureau of Agricultural and Industrial Chemistry; later it was obtained on contract from Texas Research Foundation.

plied within lanolin rings on primary leaves of bean plants in the manner described above. During the following 6-day period, 2,4-D was more effective in inhibiting bud growth than was 2,4-D-5-I (Table 1).

The herbicidal properties of the 2,4-D-5-I were compared with those of 2,4-D. 2,4-D-5-I was dissolved to make a concentration of 1000 ppm in water containing 0.5% of Tween 20. An equal concentration of 2,4-D was prepared in the same manner. The mixtures were sprayed separately on two groups of bean seedlings. Within 6 days, all plants treated with the 2,4-D were dead and there was no apparent increase in the size of the plants. In contrast, plants sprayed with 2,4-D-5-I survived for 11 days following treatment. During this period there was extensive cell proliferation, mainly in sensitive tissues of the stems. Terminal bud growth was, however, completely checked by 2,4-D-5-I, and the plants finally died. Thus, 2,4-D-5-I was somewhat less toxic to bean plants than was 2,4-D under the test conditions, since the latter compound killed the plants more quickly and caused less cell proliferation.

of both 2,4-D and 2,4-D-5-I dissociate within the plant to give two effective phenoxy components for each molecule.

Dissociation of the cupric salt of 2,4-D-5-I was not indicated by results of the present tests. It should be stressed that the activity of a compound is determined by such factors as rate of absorption and translocation as well as by dissociation.

In other experiments three additional derivatives of 2,4-D-5-I, namely the triethanoleamine salt, the *n*-butyl ester, and the isopropyl ester, were tested for growth-regulating activity. These had growth-regulating properties similar to the other iodo compounds tested.

In summary, 2,4-dichloro-5-iodophenoxyacetic acid was found to have plant growth-regulating properties. Fourteen derivatives that included salts and esters of this acid also brought about plant growth-regulating effects. The ethyl ester was the most effective of these iodo compounds under the test conditions. 2,4-Dichlorophenoxyacetic acid was more active, both from the standpoint of growth inhibition and with respect to herbicidal properties, than

TABLE 2

GROWTH-INHIBITING EFFECTS OF 10 MICROGRAMS OF 2,4-D COMPARED WITH THAT OF EQUIMOLAR CONCENTRATION OF 2,4-D-5-I AND DERIVATIVES

Compound	Mg used per 10 ml for equimolar concentration	Average fresh wt terminal growth (g)	Percent inhibition of terminal growth
Untreated control	0.00	1.93	00
2,4-Dichlorophenoxyacetic acid	5.00	0.16*	92
Ethyl 2,4-dichloro-5-iodophenoxyacetate	8.49	0.51*	74
Calcium 2,4-dichloro-5-iodophenoxyacetate	16.55	0.54*	72
2,4-Dichloro-5-iodophenoxyacetamide	7.83	0.65*	66
Morpholine 2,4-dichloro-5-iodophenoxyacetate	9.82	0.68*	65
Methyl 2,4-dichloro-5-iodophenoxyacetate	8.17	0.73*	62
Ethylammonium 2,4-dichloro-5-iodophenoxyacetate	8.85	0.79*	59
Ammonium 2,4-dichloro-5-iodophenoxyacetate	8.24	0.81*	58
Potassium 2,4-dichloro-5-iodophenoxyacetate	8.70	0.83*	57
2,4-Dichloro-5-iodophenoxyacetic acid	7.85	0.85*	56
Diethylammonium 2,4-dichloro-5-iodophenoxyacetate	9.50	0.85*	56
Sodium 2,4-dichloro-5-iodophenoxyacetate	8.35	0.91*	53
Cupric 2,4-dichloro-5-iodophenoxyacetate	17.09	1.00*	48

* Significantly less than the control at 1% level, 0.65 g being required for significance at this level.

The growth-inhibiting effect of 2,4-D-5-I and 11 of its derivatives was compared with that of 2,4-D at equal molar concentrations (Table 2), the treatments being applied to bean seedlings as previously described.

The relatively high activity of the calcium salt is thought to be due, in part at least, to dissociation of the compound within the plant. An experiment with 2,4-dichlorophenoxyacetic acid was carried out to test this theory. By using methods similar to those described above, it was determined that the activity of 2,4-D in inhibiting terminal growth of bean plants was approximately equal to that of the calcium salt of 2,4-D when compared on an equivalent weight basis. Applied at an equimolar concentration the activity of the calcium salt was roughly twice that of 2,4-D, which indicates that the calcium salts were any of the iodo compounds tested. Evidence was obtained indicating that the calcium salt of 2,4-dichlorophenoxyacetic acid and 2,4-dichloro-5-iodophenoxyacetic acid dissociate within the plant. Although 2,4-dichlorophenoxyacetic acid differs from the iodo compounds with respect to degree of herbicidal activity, the method of absorption and translocation of these compounds appears to be similar.

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Centrifugation at Temperatures Near Freezing

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During the course of an investigation of isolated chloroplasts, it was necessary to centrifuge material at temperatures just above freezing (2°–5° C). A refrigerated centrifuge was not available but the procedure described here was found useful in maintaining the material at these temperatures during centrifugation.

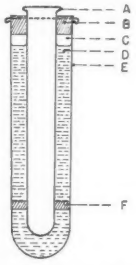

FIG. 1. *A*, 15-ml cellulose nitrate centrifuge tube. *B*, rubber washer. *C*, air space to allow for expansion of water on freezing. *D*, water in jacket. *E*, 50-ml cellulose nitrate centrifuge tube. *F*, rubber washer.

The double-walled plastic centrifuge tube shown in Fig. 1 was placed, together with a standard 50-ml metal tube holder, in the freezing compartment of a refrigerator and the water jacket was frozen solid before using.

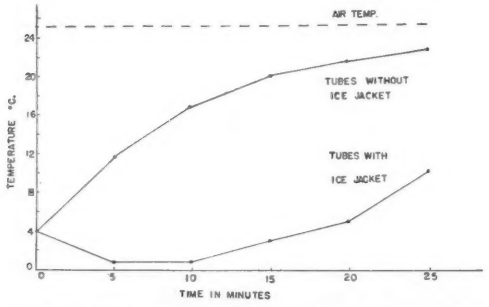

FIG. 2. Efficiency of the ice jacket in maintaining centrifuge tubes at low temperatures.

It was possible to maintain liquid samples in these tubes at 1°–5° C for 20 min of continuous centrifugation at a speed of 4200 rpm in a fixed angle centrifuge. The outside air temperature was 25° C. An initial chilling of the entire centrifuge head in the refrigerator did not prevent tubes without the ice jacket from reaching 12° C in 5 min (Fig. 2).

When the precipitate from the centrifugation was resuspended in the same tube and centrifuged again, it was found desirable to freeze the partly melted water-ice jacket by placing the tube in an ice-salt freezing mixture. By this technique a precipitate could be repeatedly washed and centrifuged at temperatures near freezing.

Thicker ice jackets can be made with larger-sized tubes. A permanent jacketed tube was made by replacing the rubber washers with plastic disks and sealing the top disk in place after adding water to the jacket.

Effects of Precipitates Formed by Insulin with Hyaluronic Acid and Mucoid from Vitreous Humor in Depressing Blood-Sugar Levels

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We have found that when insulin at pH 5–6 is added to dilutions of either hyaluronic acid or mucoid isolated from vitreous humor of ox eyes, precipitates are formed. We tested these at once for their effects on the blood-sugar level in the intact animal. Vitreous humor from fresh ox eyes was used, both to prepare hyaluronic acid (Meyer and Palmer methods [3]) and to obtain the mucoid (Suzuki method [5] modified by two of the writers [1]).

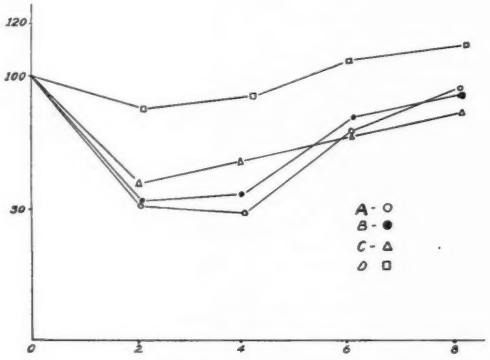

FIG. 1. Changes in the blood-sugar level following the injection of solution A, B, C, and D. Abscissas represent hours; ordinates, blood-sugar mg/100 ml.

Lilly insulin, 40 I.U./ml, with 2.0 mg phenol/ml, diluted with distilled water to 3.65 I.U./ml (*solution A*), was used as a standard of activity. Seven rabbits received intradermally 0.5 I.U./kg of this solution.

The precipitate formed by adding 1 ml of the original insulin to 10 ml of a solution of hyaluronic acid (100 μg/ml), kept for 12 hr in the refrigerator, centrifuged, rinsed with water, and resuspended in the original volume, was called *solution B*. Of this, 0.14 ml (theoretically equivalent to the values of insulin used) was given intradermally to two rabbits.

Solution C was prepared by adding 1 ml of the original insulin solution to 10 ml of a mucoid dilution (about 50 μg/ml), centrifuging the white flocculate formed, wash-

TABLE 1

TOTAL NITROGEN, PHENYLALANINE, AND HISTIDINE IN MUCOID, HYALURONIC ACID, INSULIN, INSULIN-MUCOID, AND INSULIN-HYALURONIC ACID

Materials analyzed	Nitrogen, %	Phenylalanine, %	Histidine, %
Vitreous humor mucoid ...	12.35	3.19	2.45
Hyaluronic acid	3.73	...	...
Insulin-mucoid precipitate .	13.97	6.45	3.87
Insulin-hyaluronic acid precipitate	12.86	...	...
Insulin	14.62	7.25	4.40

ing and resuspending it in the original volume of water. Of this suspension 0.14 ml/kg was injected intradermally in seven rabbits.

The supernatant fluid in solution C, after centrifuging, was separated and called *solution D*. From it 0.14 ml/kg was injected in a control rabbit.

Blood-sugar determinations were made (by the method of Hoffmann [*2*]) before each injection and every 2 hr afterwards, and the results were plotted together with relation to time, as shown in Fig. 1. From this, it will be seen: (1) that there was no noticeable difference between the effects of solutions A and B; (2) that solution C, although slightly less effective during the first 2 hr than A or B, exerted a more prolonged effect, and for this reason the original sugar values return more slowly to their former level; (3) that solution D was still active, but in a much lower degree.

We have planned an investigation of the nature of the precipitates formed. A priori, one is tempted to identify those formed by hyaluronic acid, mucoids or mucins, and described by Meyer and Palmer, as mere artifacts. For the time being we have conducted analysis of only the total nitrogen content (by micro Kjedahl) in the two above-mentioned precipitates, and also in mucoid, hyaluronic acid, and insulin (the last precipitated from the original solution by alcohol ether). Phenylalanine and histidine in the precipitates, insulin, and mucoid were determined by Stokes *et al.* (*4*) by microbiological method. Table 1 reports the results from duplicate determinations.

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Spawning of Oysters at Low Temperatures

Victor L. Loosanoff and Harry C. Davis

Milford Laboratory, U. S. Fish and Wildlife Service, Milford, Connecticut

The assumption by Churchill (*1*), Galtsoff (*2*), Nelson (*5*), and others that 20.0° C is the minimum temperature for spawning of the oyster (*Ostrea virginica*) in nature was first questioned when it was established that oysters in Long Island Sound were spawning at temperatures ranging from 16.4° to 18.5° C (*4*). Systematic observations conducted since then by one of us have shown that in some years not only spawning but also larval metamorphosis, or, as it is commonly called, setting, took place in Long Island Sound before the temperature reached 20° C. Reports of these observations are in the official files of the U. S. Department of the Interior, Fish and Wildlife Service. However, this evidence was not corroborated by laboratory observations.

The first direct observation on mass spawning of laboratory oysters at a comparatively low temperature was made in May 1949. These oysters were kept for a period of 90 days in trays with running sea water, the temperature of which was steadily maintained at 15° ± 1° C. Because samples of gonads taken toward the end of the 90-day period began to show that the oysters were discharging some spawn, and because examinations of the bottom deposit disclosed fertilized eggs, it was decided to add sperm and egg suspension to one tray to see if the oysters would respond to the stimulation and begin spawning. Within a minute after addition of the suspension, the first male began to spawn. He was soon followed by two other males, and after 5 min three females were also spawning. Later on more oysters of both sexes began to spawn, and finally, after about 25 min almost all 20 oysters were spawning, and continued to do so for about 2 hr.

Addition of sperm and eggs to three other trays also induced mass spawning, with oysters of both sexes discharging large quantities of eggs or sperm. Throughout spawning, the water temperature in all trays remained steadily at 15.8° C. Fertilized eggs were collected and cultured; they showed normal development.

Obviously, spawning of a certain portion of the oyster population in northern waters may begin at temperatures much lower than 20° C. It is possible that the temperatures prevailing during the period of gonad maturation may determine the temperature at which the first spawning will take place.

As Thorson (*6*) points out, many marine invertebrates may have ripe sexual products outside the breeding period, sometimes months ahead of spawning. In our waters, such is the case in *Venus mercenaria*, which possesses ripe sperm and morphologically mature ova in November, although it does not spawn until the following July or August (*3*). Therefore, a distinction should be made between the temperature high enough to permit maturation of sexual products and the temperature at which spawning is possible. Sometimes such temperatures are several degrees apart. However, as our laboratory observations indicate, by conditioning oysters for a long period at a temperature just high enough for maturation of the gonads it is possible to induce spawning without an increase in temperature, thus bringing together, or to virtually the same level, the temperatures needed for ripening of gonads and for spawning.

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On Interspecific Hybridization in *Ostrea*

Harry C. Davis[1]

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In view of numerous attempts to introduce the Japanese oyster, *Ostrea gigas*, to the Atlantic coast it is important to determine whether this species will cross with the native oyster, *Ostrea virginica*, which is found from Massachusetts to Florida.

Galtsoff and Smith (*2*) made some preliminary observations in determining whether these two species would cross. They reported that "Eggs of both species were easily fertilized by either sperm, the ensuing development resulted in apparently normal straight hinge larvae. Comparison with controls showed no increased mortality among the hybrids." Since their observations terminated at the very early straight-hinge stage, it remained undetermined whether the hybrid larvae would continue to develop normally and would finally metamorphose. The present study was undertaken to provide an answer to this question, using the methods now standard at Milford Laboratory for rearing larvae to metamorphosis.

Unfertilized eggs and active spermatozoa of *O. virginica* and *O. gigas* were obtained from mature individuals and allowed to stand suspended in aerated sea water for a short time. The egg suspensions were divided into six equal portions, after an examination had shown that they were free of casual spermatoza. To each of three portions of *O. virginica* eggs, a small volume of *O. virginica* spermatozoa was added, and the remaining three portions received the same quantity of spermatozoa from *O. gigas*. Thus, triplicate cultures of *O. virginica* ♀ × *O. virginica* ♂ and *O. virginica* ♀ × *O. gigas* ♂ were prepared. Eggs from *O. gigas* were handled in the same manner, giving triplicate cultures of *O. gigas* ♀ × *O. virginica* ♂, and *O. gigas* ♀ × *O. gigas* ♂. This assured that eggs and spermatozoa used in hybrid crosses were from the same source and therefore were equal in viability to those used in the control nonhybrid cultures. All 12 groups of fertilized eggs were then cultured under identical conditions. The experiment was repeated three times with consistent results.

In general, hybrid larvae develop quite normally to the straight-hinge veliger stage. However, contrary to the observations of Galtsoff and Smith (*2*), in most of the hybrid cultures of *O. virginica* ♀ × *O. gigas* ♂ the proportion of larvae developing to the straight-hinge stage was appreciably lower than for the corresponding control nonhybrid cultures. Furthermore, in all hybrid cultures a very high mortality became apparent about the sixth day, and almost all the larvae were dead by the tenth day. This heavy mortality in such a short period was especially striking because the hybrid larvae were entirely normal in appearance and vigorous in behavior until the onset of mortality.

The control nonhybrid cultures of *O. virginica* and *O. gigas*, on the other hand, grew quite normally, with no undue mortality, and all cultures were reared to the setting stage. The *O. virginica* cultures reached metamorphosis in 19–25 days, whereas most of the *O. gigas* cultures required 26 to 27 days to metamorphose.

As shown by these experiments, hybrid larvae died within 6–10 days after fertilization, although the nonhybrid cultures grown under the same conditions developed normally to metamorphosis. The mortality of hybrid larvae was apparently due to a lethal combination of inherited factors, that did not become active until a specific stage of development, which most larvae attained in 6–10 days, although the time may be dependent on temperature and thus may vary considerably. The possibility is not excluded, however, that in some rare cases a few of the hybrid larvae, characterized by a special genetic complex, might survive to metamorphosis or even to maturity.

Crosses of *O. virginica* with *O. lurida* were also tried, but the experiments were necessarily confined to attempts to fertilize *O. virginica* eggs with *O. lurida* spermatozoa, because many individuals of the latter species, in addition to being larviparous, are also hermaphroditic, and one cannot, therefore, be certain of exclusion of their spermatozoa. It was possible, however, to find many individuals that appeared to be true males at the time and these were stripped to obtain spermatozoa for the experiments in which active *O. lurida* spermatozoa were added to unfertilized eggs of *O. virginica*. Although the mixture of eggs and spermatozoa was held, in some cases, for as long as 8 hr, no fertilization occurred. Observations also showed that the spermatozoa of *O. lurida* did not interfere with later fertilization of these eggs by spermatozoa of their own species, even when this addition of *O. virginica* spermatozoa was as late as 8 hr after the unsuccessful attempt to fertilize the eggs with spermatozoa from *O. lurida*. Apparently the *O. lurida* sperm does not even enter the *O. virginica* egg, since it does not cause formation of a fertilization membrane or interfere in any way with the later fertilization of the egg by *O. virginica* spermatozoa. These results closely parallel those of Bouchon-Brandely (*1*), who also attempted to cross a larviparous species (*O. edulis*) with an oviparous species (*O. angulata*) and reported that there was no "evidence of successful fertilization or of development."

[1] The writer wishes to express his appreciation to Mr. C. E. Lindsay, senior biologist, Washington Department of Fisheries, for shipping us the *Ostrea gigas* and *Ostrea lurida* used in these experiments.

References

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Association Affairs

Laurentian Hormone Conference September 10–15, 1950

The Laurentian Hormone Conference of the AAAS will hold its 1950 meeting at the Forest Hills Hotel, Franconia, New Hampshire, September 10–15. Attendance at this conference is limited by the accommodations available at the hotel, but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee consists of Gregory Pincus, The Worcester Foundation for Experimental Biology, chairman; R. W. Bates, E. R. Squibb and Sons; R. D. H. Heard, McGill University; A. D. Odell, Charles E. Frosst and Company; E. C. Reifenstein, Jr., Sloan-Kettering Institute; and A. White, School of Medicine, University of California at Los Angeles.

Application blanks for attendance at the Conference may be obtained from Dr. Pincus, 222 Maple Avenue, Shrewsbury, Massachusetts. Applications to be considered by the Committee must be received by *May 20*.

The following program has been arranged:

I. Gonad Development and Function

Sunday evening, September 10

"Embryogenesis of the adrenal and the reproductive glands,"

Dr. Emil Witschi, The State University of Iowa.

Monday morning, September 11

"Mammalian spermatogenesis,"

Warren O. Nelson, The State University of Iowa.

Monday morning, September 11

"Oogenesis in the mature ovary,"

S. Zuckerman, University of Birmingham, England.

II. The Use of Isotopes in Hormone Problems

Monday evening, September 11

"Studies in the metabolism of steroid hormones,"

T. F. Gallagher, Sloan-Kettering Institute.

Tuesday morning, September 12

"Biosynthesis of steroids,"

Konrad Bloch, The University of Chicago.

III. Hormones and Metabolism

Tuesday morning, September 12

"Parameters of metabolic problems,"

Dwight J. Ingle, The Upjohn Company.

Tuesday evening, September 12

"Metabolic processes influenced by the growth hormone,"

F. J. Young, Biochemical Laboratory, Cambridge, England.

Wednesday morning, September 13

"The problem of adrenal androgens in man,"

Allan T. Kenyon, The University of Chicago.

IV. Chemistry, Physiology, and Clinical Endocrinology of the Adrenal Cortex

Wednesday morning, September 13

"Chemistry of adrenal cortex steroids,"

P. L. Julian, The Glidden Company.

Thursday morning, September 14

"The nature of adrenal secretory activity."

Oscar Hechter, The Worcester Foundation for Experimental Biology.

Thursday morning, September 14

"The adrenal cortex in salt and water metabolism."

Robert Gaunt, Syracuse University.

Thursday evening September 14

"A consideration of the role of the adrenal cortex in the regulation of certain metabolic processes."

Frank L. Engel, Duke University.

Friday morning, September 15

"Observations on the physiological effects of cortisone and ACTH in man,"

R. G. Sprague, M. H. Power and H. L. Mason, The Mayo Clinic.

V. Hormone Therapy in Cancer

Friday morning, September 15

"Experimental studies with ACTH and cortisone in patients with neoplasma,"

O. H. Pearson, Sloan-Kettering Institute.

Friday evening, September 15

"Steroid hormone therapy in cancer,"

I. T. Nathanson, Massachusetts General Hospital.

Report of the Special Committee on the Civil Liberties of Scientists

The conclusions and recommendations of the special committee of the American Association for the Advancement of Science on Civil Liberties of Scientists, of which Maurice B. Visscher is chairman, were published in *Science*, August 19, 1949. As there have been numerous requests for the complete report, the Executive Committee has voted to make it available at cost.

Although there is general recognition of the actual and potential dangers of national authoritarianism to scientific research and freedom of expression, it is inevitable that there should be wide differences of opinion among the more than 44,000 members of the AAAS regarding what is said in the name of the Association and how it is said. The special committee worked long and conscientiously on the report, accepted criticisms and suggestions, made sincere attempts to harmonize differences of opinion, and presents the report in the hope that its readers will recognize that it is an attempt to present the problems and to suggest remedies in the spirit of President Tru-

man's statement, "Continuous research by our best scientists is the key to American leadership and true national security. This work may be made impossible by the creation of an atmosphere in which no man feels safe against the public airing of unfounded rumors, gossip, and vilification."

Copies of the report may be obtained by addressing the Business Office, AAAS, 1515 Massachusetts Avenue, N.W., Washington 5, D. C. The price is $1.00 for single copies; 90¢ each for 5 or more copies; 80¢ each for 20 or more copies. Checks or money orders must accompany requests. Orders should be sent in promptly. Copies of the report will be available about June 1.

E. C. STAKMAN

Chairman, Executive Committee

A New Association Building

At the New York meeting of the Association, President Adams reported to the Council on the work of the Committee on a New Association Building. The Council instructed the Committee to proceed with the formulation of plans to secure funds for construction and designs for an appropriate structure. The Committee consists of Roger Adams, chairman, Paul Klopsteg, Howard Meyerhoff, E. C. Stakman, and Warren Weaver.

The Committee's thinking has centered on the effective utilization of the Scott Circle property, which was acquired by purchase in 1946. Zoning regulations will permit the construction of an eight-story building on the site, though there are several restrictions on the uses to which such a building can be put. The first decision confronting the Committee, therefore, was whether to fill the property with a large building in which rentals would be limited to nonprofit organizations, or whether to build solely with the current needs and potential growth of the Association in mind.

This question was discussed at length by the Executive Committee on March 25, and the second of the two choices was strongly favored for several reasons: The cost of a large structure will exceed $1,300,000, whereas a more modest building can be erected for less than $500,000. Financing the latter project would be a simpler problem and, presumably, could be accomplished within a short period of time. Furthermore, there is little prospect of finding a sufficient number of homeless nonprofit organizations to fill a large structure, and zoning regulations preclude other types of tenants.

Although one or more substantial gifts toward the erection of a new building might persuade the Executive Committee to reconsider its decision, subsequent planning will focus on a modest structure large enough to house the Association through another quarter of a century of growth and so designed as to permit additions when this growth is achieved. Meanwhile, whatever space is available in excess of requirements could be utilized to provide temporary shelter for other organizations, such as the American Psychological Association, which now occupies the third floor at 1515 Massachusetts Avenue.

HOWARD MEYERHOFF

Administrative Secretary

Scientific Book Register

Natural Regions of the U. S. S. R. L. S. Berg; trans. from Russian by Olga Adler Titelbaum. New York: Macmillan, 1950. 436 pp. $10.00.

Races: A Study of the Problems of Race Formation in Man. Carleton S. Coon, Stanley M. Garn and Joseph B. Birdsell. Springfield, Ill.: Charles C. Thomas, 1950. 153 pp. $3.00.

Human Fertility and Problems of the Male. Edmond J. Farris. White Plains, N. Y.: Author's Press, 1950. 211 pp. $5.00.

Fundamentals of Organic Chemistry: A Brief Course for Students concerned with Biology, Medicine, Agriculture, and Industry. James Bryant Conant and Albert Harold Blatt. New York: Macmillan, 1950. 413 pp. $4.00.

Highways in Our National Life: A Symposium. Jean Labatut and Wheaton J. Lane, Eds. Princeton, N. J.: Princeton Univ. Press, 1950. 506 pp. $7.50.

Criminology: A Cultural Interpretation. Rev. ed. Donald R. Taft. New York: Macmillan, 1950. 704 pp. $5.50.

Botany: An Introduction to Plant Science. Wilfred W. Robbins and T. Elliot Weier. London: Chapman & Hall; New York: John Wiley, 1950. 480 pp. $5.00.

Cosmological Theory. 2nd ed. G. C. McVittie. London: Methuen and Company; New York: John Wiley, 1949. 103 pp. $1.50.

Introducing the Insect. F. A. Urquhart. New York: Henry Holt, 1950. 287 pp. $5.00.

Counting Tubes: Theory and Applications. S. C. Curran and J. D. Craggs. London, England: Butterworths Scientific Publs.; New York: Academic Press, 1949. 238 pp. $5.50.

Semimicro Qualitative Analysis. William C. Oelke. Boston: D. C. Heath, 1950. 377 pp. $3.75.

Textbook of Bacteriology. 2nd ed. Joseph M. Dougherty and Anthony J. Lamberti. St. Louis, Mo.: C. V. Mosby, 1950. 491 pp. $5.75.

L'Image du Monde. Gabriel Monod-Herzen. Paris, France: Librairie Gallimard, 1950. 197 pp. 320 fr.

Photoelasticity: Principles & Methods. H. T. Jessop and F. C. Harris. New York: Dover Publs., 1950. 184 pp. $3.50.

College Physics. 3rd ed. C. E. Mendenhall *et al.* Boston: D. C. Heath, 1950. 657 pp. $5.50.

Arithmetic for Colleges. Harold D. Larsen. New York: Macmillan, 1950. 275 pp. $3.75.

Aerials for Centimetre Wave-lengths. D. W. Fry and F. K. Goward. New York: Cambridge Univ. Press, 1950. 172 pp. $3.50.

News and Notes

Paris Conference on the Fundamental Constants of Astronomy

G. M. Clemence

U. S. Naval Observatory, Washington, D. C.

An international conference on the fundamental constants of astronomy was held in Paris, March 27–April 1, under the auspices of the Centre National de la Recherche Scientifique. Nineteen delegates from six countries participated; those from the U. S. were Dirk Brouwer, G. M. Clemence, and H. R. Morgan.

Nine resolutions were adopted, six of them in the form of recommendations addressed to the International Astronomical Union. There was unanimous agreement on the undesirability of making any changes in values of fundamental constants that are now used internationally in the calculation of astronomical ephemerides. Although most of these have now been in use for 50 years, and although the values of some of them are known to be inconsistent with one another, it is not yet clear what values would be preferable to adopt in their place, and it seemed impossible to make any revision of the system that might not require further revision during the next few years. An important resolution concerned the fundamental unit of time, which heretofore has been based on the rotation of the earth. The rate of rotation is variable, being affected by secular, seasonal, and other changes. It was proposed to adopt instead the mean period of the earth's sidereal revolution around the sun.

Other resolutions dealt with the methods used for predicting the positions of the sun, moon, and planets. For the planets Mars, Jupiter, Saturn, Uranus, Neptune, and Pluto, international adoption was recommended of the methods newly developed under sponsorship of the Office of Naval Research, at Yale University, the U. S. Naval Observatory, and the Watson Scientific Computing Laboratory.

The conference was marked throughout by a unanimity of opinion that was quite astonishing in view of the legitimate differences that might reasonably be expected on the highly technical matters discussed. No delegate dissented from any of the actions taken and most were by unanimous agreement.

The proceedings of the conference will be published in full in the *Bulletin Astronomique.*

ONR Cryogenics Conference

W. T. Ziegler

Georgia Institute of Technology, Atlanta

A cryogenics conference sponsored by the Office of Naval Research, Department of the Navy, was held at the Georgia Institute of Technology, Atlanta, Georgia, March 20–21. More than 60 scientists from 24 institutions engaged in low temperature research attended the meetings, at which recent developments in this field were discussed. Sessions were devoted to cryomagnetics, superconductivity, thermal properties of matter, and miscellaneous problems. Features of the meeting were two invited review papers, one by J. H. Van Vleck of Harvard University, entitled "The Present Status of Cryomagnetics," and one by F. G. Brickwedde of the National Bureau of Standards, entitled "Thermal Properties at Low Temperatures."

The conference provided an opportunity for the presentation and discussion of new results and for the exchange of ideas and techniques in the field of low temperature research. It also indicated the need for better coordination between different laboratories and at the same time provided an opportunity for such coordination. In cryomagnetics, for instance, the need was indicated for studies by different investigators of the same paramagnetic salt, preferably in the form of single crystals, since such studies would provide more adequate data for comparison with theory. New developments in the design and operations of magnets, thermal switches, and moving mechanical contacts for use in cryomagnetic experiments attested to the active interest in equipment design in this field.

Among the interesting experimental results reported in the field of superconductivity were those on the mercury isotopes. Experiments on these isotopes indicated that the normal transition temperature decreased by 0.01° per unit increase in mass number. A discussion was presented on the difficulty of preparing emulsions containing spherical lead, tin, and mercury particles of uniform size, together with the uncertainty introduced in the value of λ (the field penetration depth) calculated from the magnetic susceptibility, because of clustering of the particles; this indicated the need for caution in using the results of such experiments to test the London theory of field penetration.

Liquid helium came in for considerable discussion. Papers were presented on the results of new measurements of the thermal conductivity of helium I, the dielectric constant of helium I and II, the superfluidity of adsorbed helium II films the λ-points of He^3-He^4 mixtures, and the mechanical detection of second sound in helium II by means of the Rayleigh Disk. The results of the experiments employing the Rayleigh Disk lend an additional independent verification of the two-fluid hypothesis for liquid helium II.

The thermal conductivities of copper, aluminum, tin, 18–8 type stainless steel, inconel, and monel at liquid helium temperatures were reported. At 2.6° K the thermal conductivities of stainless steel and inconel were found to be about one milliwatt/cm/deg and that of monel about twice as great. These data will be of particular value to designers of apparatus for use in this temperature range.

Measurements on the electrical resistance of gold, magnesium, and aluminum were reported; it was shown that both gold and magnesium exhibit a resistance minimum, whereas no minimum was found in aluminum down to 2° K.

Still other papers dealt with the vapor pressure and critical constants of HD and D_2; the dew points of mixtures of H_2, HD, and D_2; the Hall effect and magnetoresistance of germanium at helium temperatures; dielectric and electrocaloric properties of barium titanate; and design calculations for a small Joule-Thomson helium liquefier.

The conference included an inspection of the Georgia Tech Low Temperature Laboratory, in which an extensive research program is being conducted under the sponsorship of the Office of Naval Research, under the direction of the writer.

Leaders in arranging this conference were I. Estermann, professor of physics at the Carnegie Institute of Technology, Pittsburgh, Pennsylvania, and the writer.

David D. Keck, a member of the Carnegie Institution of Washington's staff at Stanford University, has been appointed head curator of the New York Botanical Garden, Bronx Park, effective January 1, 1951.

Jesse E. Edwards, assistant professor of pathologic anatomy, Mayo Foundation, University of Minnesota, will deliver the 26th Ludvig Hektoen Lecture of the Frank Billings Foundation of the Chicago Institute of Medicine on May 26. His subject will be "Structural Changes of the Pulmonary Vascular Bed and their Functional Significance in Congenital Cardiac Disease."

Ira M. Freeman, associate professor of physics at Rutgers University, has been granted a year's leave of absence to enable him to undertake a special assignment in the Division of Natural Sciences of Unesco in Paris.

Avery S. Hoyt has been appointed chief of the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture, to replace **Percy N. Annand,** who died March 29. Dr. Hoyt, who served as acting chief of the bureau after Dr. Annand's death, has been associated with the Agriculture Department since 1912.

Thomas A. C. Rennie has been named professor of psychiatry at Cornell University Medical College. Dr. Rennie is attending psychiatrist at Payne Whitney Psychiatric Clinic, New York Hospital and, as consultant at the Kingsbridge and Montrose Veterans Administration hospitals, has taken part in the organization of the psychiatric training programs in these institutions.

William S. Stone, (Col. U.S.A.), has been appointed commandant of the Army Medical Department Research and Graduate School, replacing **Elbert DeCoursey** (Col. U.S.A.), who will take over direction of the Armed Forces Institute of Pathology. Col. Stone, who served as chief of preventive medicine in the North African campaign and as special advisor to the Health and Welfare Division of the U. S. Military Government in Germany, has been chairman of the Army Surgeon General's Medical Research and Development Board since 1946.

James D. Majarakis, instructor in the Department of Surgery of the University of Illinois College of Medicine, has been appointed executive officer of the 427th General Hospital, a 1,000-bed U. S. Army reserve unit which is sponsored by the university.

Visitors to U. S.

David Katz, director of the Psychological Institute, University of Stockholm, has been named the second Hitchcock Lecturer at the University of California for 1949–50. Dr. Katz, who is well known for his work on the perception of color and for his studies of the sense of touch, will give a series of public lectures at Berkeley, May 9–31.

Members of the University of Michigan's visiting faculty who will lecture during the Institute on the Near East, June 26–August 18, include **Emile Beneviste,** Collège de France; **L. P. Elwell-Sutton,** language supervisor for the British Broadcasting Corporation; and **George Hourani,** Balliol College, Oxford. Information regarding the lecture schedule may be obtained from Dr. George Cameron, Department of Near Eastern Studies, 2023 Angell Hall, University of Michigan, Ann Arbor.

Recent visitors at the National Bureau of Standards were **L. Mullins,** member of the British Rubber Producers Research Association, Welwyn Garden City, England; **T. F. Van Oss,** representing the Dutch Government, **G. J. Van Veersen,** organic chemist, Plastics Institute, Delft, Holland; **Kristen Bo,** chemist, Jacob Holm and Sons, Copenhagen, Denmark; **N. E. Dobbins,** director of research, Vereeniging Brick and Tile Company, Vereeniging, South Africa.

D. J. van Lennep, professor of diagnostic psychology at the University of Utrecht, will be visiting professor at Pennsylvania State College from August 14 to September 1. He plans to arrive here early this summer and will be available for lectures.

Grants and Awards

The Albert and Mary Lasker Awards for 1949 were presented to William L. Laurence and Herbert and Dixie Yahraes at the semiannual meeting of the National Association of Science Writers, held April 25 in Washington, D. C. The awards, the first to be made in the field of journalism by the Lasker Foundation, were established to encourage the writing and publication

in newspapers and magazines of outstanding articles on public health improvement and the prolongation of life, especially through medical research and public health programs.

Mr. Laurence received the newspaper award for a series of articles published in the *New York Times* on the development of cortisone and ACTH. He has been a science writer on the *Times* for more than 20 years and has won two Pulitzer prizes. Mr. and Mrs. Yahraes received the magazine award for their article "Our Daughter Is an Epileptic," published in *Collier's*, November 26, 1949. Mr. Yahraes has twice received honorable mention for the Westinghouse Science Writing Award.

Each of the winners received $500 and a statuette of the Winged Victory of Samothrace, symbolizing triumph over death and disease.

Leason Heberling Adams, geophysicist and physical chemist, has been named the twelfth recipient of the **William Bowie Medal**, awarded annually by the American Geophysical Union. Dr. Adams is director of the Carnegie Institution of Washington's Geophysical Laboratory.

Fellowships

Forty-five Shell Oil Company graduate fellowships are being offered for 1950–51 at 31 colleges and universities, in the fields of chemistry, chemical engineering, geology, geophysics, mechanical and petroleum engineering, physics, and plant science. The fellowships provide tuition and fees, a fixed stipend of $1,200, and a fund of $300 for research equipment and supplies. The respective schools are responsible for selection of fellows, and the departments concerned determine the specific research. Further information may be obtained from the Shell Fellowship Committee, 50 West 50th Street, New York City.

The Jessie Horton Koessler Fellowship of the Institute of Medicine of Chicago for research in biochemistry, physiology, bacteriology, or pathology will be available September 1. The stipend is $500 a year with the possibility of renewal for one or two years. Applications must be approved by the head of a department in these fields or by the director of a research institute or laboratory in Chicago, and must stipulate that the recipient of the fellowship shall be given adequate facilities for carrying out the proposed research. Applications should be sent in quadruplicate to Dr. Paul R. Cannon, Chairman of the Committee on the Jessie Horton Koessler Fund, 950 East 59th Street, Chicago, before *July 1*.

Colleges and Universities

The Massachusetts Institute of Technology and **Barnard College at Columbia University** each received last month a gift of one million dollars from John D. Rockefeller, Jr. The gifts are to be used in the over-all development programs of the two colleges. Mr. Rockefeller expressed the hope that Barnard's fund would be applied ultimately to the contemplated new building which is to house the college's science departments, library, and theater.

The College of the Holy Cross, Worcester, Massachusetts, has begun construction of a new $500,000 biology building which will accommodate a working biology museum and a complete biology reference library. Photographic laboratories and darkrooms for training students in photomicrography and the preparation of lantern slides are included in the plans, in addition to a microtechnique laboratory.

Meetings and Elections

The Society of American Bacteriologists will hold its 50th general meeting at the Lord Baltimore Hotel, Baltimore, May 14–18. The scientific program will be presented before sections on general bacteriology, agricultural and industrial bacteriology, medical bacteriology and immunology, and the physiology of microorganisms. A number of round table discussions on subjects of current interest to microbiologists are planned. On Sunday evening, May 14, Detlev W. Bronk, president of Johns Hopkins University, will address the members and guests of the society at a public lecture on the subject of fundamental scientific research. It is anticipated that approximately 2,000 persons will register for the meetings.

The American Society for Engineering Education will hold its annual convention June 19–23 at the University of Washington, Seattle. More than 100 research directors and administrators from educational institutions throughout the country are expected to participate in the Engineering College Research Council conferences which will take place during the first two days and which will provide a comprehensive survey of the engineering research activities in the Pacific Northwest.

The Third Heat Transfer and Fluid Mechanics Institute, sponsored by five western universities and five technical societies, will take place June 28–30 at the Institute of Aeronautical Sciences Building, Los Angeles. Information concerning the program may be obtained from J. C. Dillon, Department of Engineering, University of California, Los Angeles 24.

The American Philosophical Society elected the following officers for 1950 at its meeting in Philadelphia on April 22: president, Edwin G. Conklin, professor emeritus of biology, Princeton University; vice presidents, Arthur H. Compton, chancellor, Washington University, St. Louis (re-elected), Herbert F. Goodrich, judge, U. S. Circuit Court of Appeals, Waldo G. Leland, director emeritus, American Council of Learned Societies, Washington, D. C.; secretaries, Jacob R. Schramm, professor of botany, University of Pennsylvania, and Richard H. Shryock, professor and director, Institute of History of Medicine, Johns Hopkins University. The society also elected 18 new members, including four from other countries.

NRC News

The Committee on Diagnosis and Pathology of Nutritional Deficiencies of the NRC's Food and Nutrition Board has prepared a report on ***Nutrition Programs for State Health Departments.*** Pointing out that malnutrition is still quite common in this country, and that many states

do not have trained physicians and biochemists in their nutritional services, the report discusses the organization, personnel, and activities which an alert state or local health agency can maintain.

Nutritional activities of a state health department are considered under the following headings: administration and policy, investigations and research, laboratory services, education and information services, dietary recommendations and standards, direct services and services for specific groups, and cooperation with physicians.

The report was recently published in *Public Health Reports* of the Public Health Service. Copies are available from the Food and Nutrition Board, NRC, 2101 Constitution Avenue, Washington 25, D. C.

A new editorial board has been appointed for ***Mathematical Tables and Other Aids to Computation,*** a quarterly journal published by the Division of Mathematical and Physical Sciences of the NRC. The members are D. H. Lehmer, professor of mathematics, University of California, Berkeley, chairman; Edward W. Cannon, assistant chief, Applied Mathematics Division, National Bureau of Standards; Cecil C. Craig, director, Statistical Research Laboratory, University of Michigan; Arthur Erdélyi, professor of mathematics, California Institute of Technology; Francis J. Murray, associate professor of mathematics, Columbia University; and John Todd, National Bureau of Standards. The retiring editor, Raymond C. Archibald of Brown University, has served the journal since its origin in 1943.

The first issue of ***United Nations Documents Index: Documents and Publications of the United Nations and Specialized Agencies,*** was published by the UN Library in February. The index lists and indexes by subject all of the documents and publications (except restricted materials and internal papers, of the UN and its specialized agencies received by the library. Each monthly issue will contain introductory material concerning distribution, sales, and other pertinent items, a list of documents and publications of the UN arranged by symbol, followed by lists for each of the specialized agencies, and a subject index in one alphabet referring to all of these documents. The subject index will be cumulated annually. Each issue will contain between 75 and 100 pages. The annual subscription is $7.50. Payments may be made in other national currencies. Orders may be placed with the Sales and Circulation Section, UN, Lake Success, New York, or Sales Section, European Office of the UN, Palais des Nations, Geneva, Switzerland, or through regular sales agents for the UN publications.

Copies of the complete papers of the **ACS Symposium on Chemical Factors in Hypertension** will be available soon. The symposium was presented at a meeting of the Division of Medicinal Chemistry in San Francisco in March, 1949. None of the papers have been published in any other journal. The 60-page volume is priced at $1 and may be obtained from the Special Publications Department, American Chemical Society, 1155 Sixteenth Street, N.W., Washington 6, D. C. as long as the supply lasts.

The Registry of Rare Chemicals, 35 West 33rd Street, Chicago 16, Illinois, lists the following wanted chemicals: zinc isopropyl xanthate, ethoxyethyl mercury chloride, 1,1,2-triiodopropane, 1,1-diiodo-2-methylpropane, 2-iodo-3-methylbutane, dihydroxymethyl ether, 2,5-dinitrophenol, 3,5-dinitrophenol, heptane-1,3-dithiol, heptane-2,4-dithiol, hexane-1,3-dithiol, 2-cyanobutadiene-1,3, 1,3-dibromohexane, 1,3-dibromopentane, 3,5-dichlorocyclohexylamine, betaine aldehyde, 2-amino-4-methylamino-6-ethylamino-1,3,5-triazine, 5-allyl-5-(1-methylbutyl) barbiturate, benzoguanamine, and solochrome cyanine.

A new laboratory for research on brucellosis has been established at the University of Wisconsin as center of a state program of control and eradication of the disease. C. A. Brandly, professor of veterinary science, is head of the brucellosis project committee at the university, which cooperates with the State Laboratory of Hygiene, the Wisconsin Board of Health, the U. S. Public Health Service, and the Wisconsin Department of Agriculture on the program. The Wisconsin legislature has given $1,500,000 for an area plan of vaccination, testing, and limited slaughter with indemnity to farmers, and an additional $50,000 for research. The State Laboratory of Hygiene is continuing its tests for human brucellosis. Nearly 900 of the 18,925 blood specimens tested there last year indicated some evidence of brucellosis.

AAAS Section G—
Botanical Sciences

Section G will hold sessions for contributed papers during the first part of the Association's 117th Meeting in Cleveland, December 26–31. A series of symposia will be presented during the latter part of the meeting. An immediate response from members who intend to present papers is necessary for purposes of program planning. Tentative titles and subject classifications should be sent to the secretary of Section G *this month.* Final titles may be submitted as late as *September 15.*

John S. Karling, Vice President
Stanley A. Cain, Secretary
Cranbrook Institute of Science
Bloomfield Hills, Michigan

Recently Received—

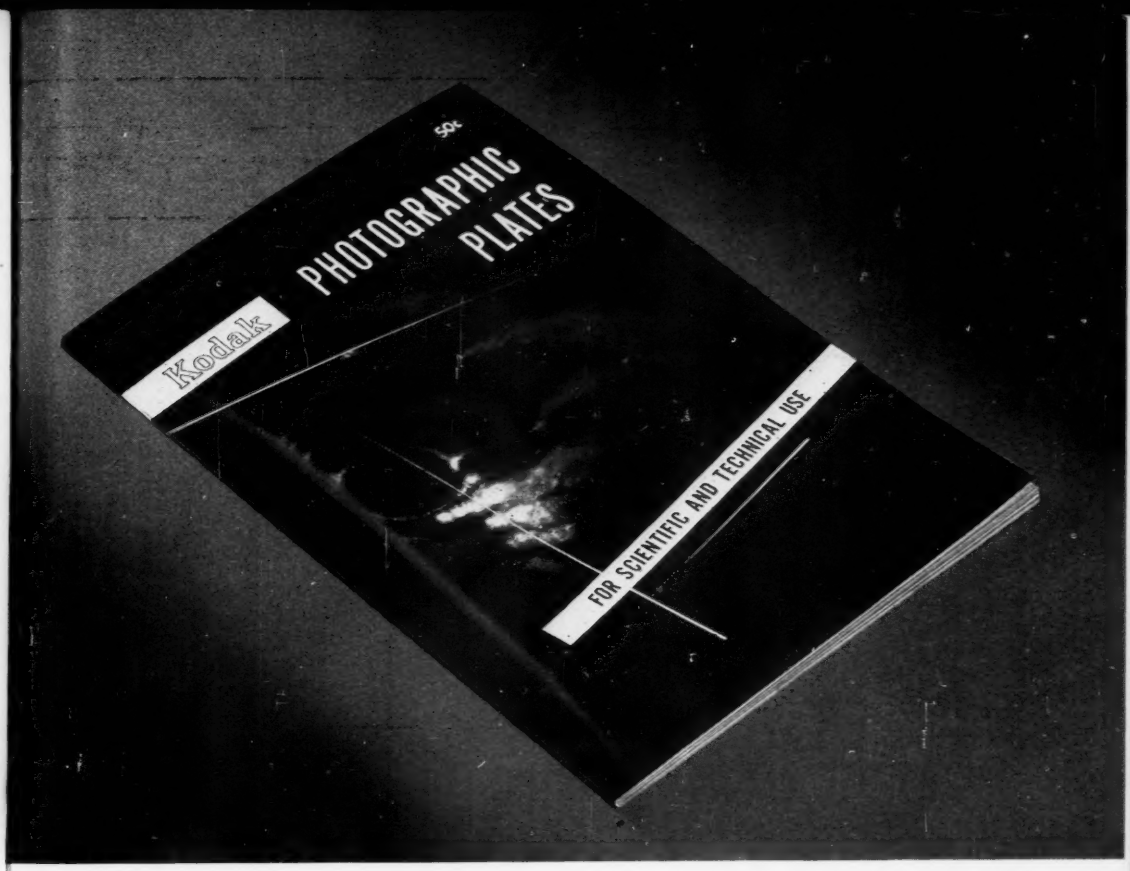
Abridged Catalog of Organic Chemicals for Laboratory Use. Smith-New York Company, Inc. Freeport, Long Island, N. Y.

Sweet Corn Trials, Mt. Carmel and Windsor, Connecticut, 1949. W. C. Galinat, Connecticut Agricultural Experiment Station, New Haven, Conn.

A New Approach to Problems Encountered in Colorimetric Absorption Analysis. A. Rouy. E. Leitz, Inc., 304 Hudson St., NYC.

Blueprint for Understanding. Institute of International Education. 2 West 45th St., NYC 19.

Report of Bureau of American Ethnology, 1948–49. Smithsonian Institution. U. S. GPO, Washington 25, D. C.



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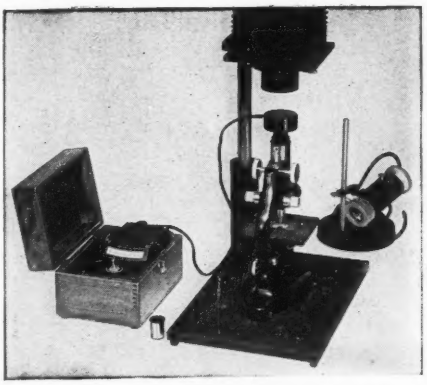

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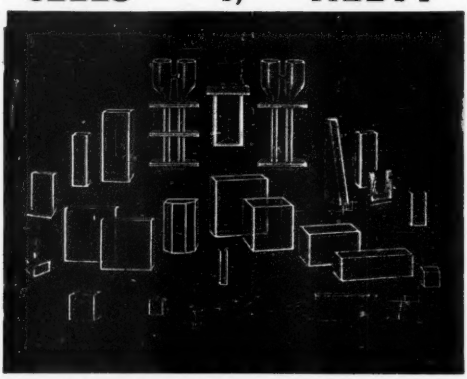

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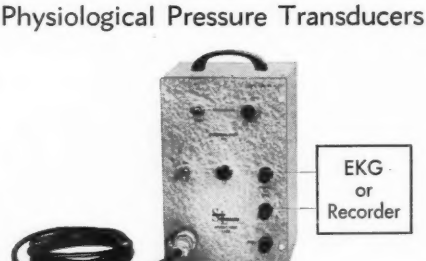

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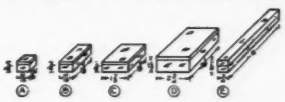

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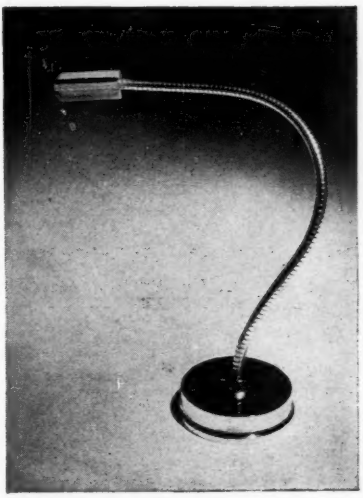
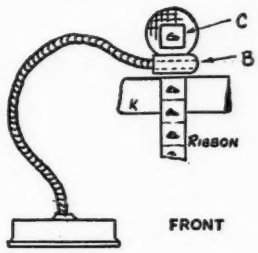


Figure 1.



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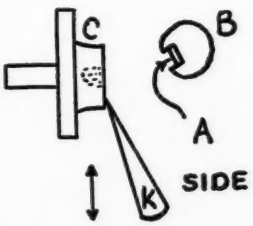

Figure 3.

The cutting of paraffin tissue sections in dry weather is often difficult or impossible due to static formation because:

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